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(54) Title: USE OF THE MUSHROOM AGARICUS BLAZEI MURILL FOR THE PRODUCTION OF MEDICAMENTS SUITABLE FOR TREATING INFECTIONS AND ALLERGIES

(57) Abstract: The present invention concerns the use of the mushroom Agaricus blazei Murill for producing a medication for combating or preventing bacterial and non-bacterial infections (e.g. parasites or virus) in a mammal as well as combating or preventing allergy in mammals. Such an infection may e.g. be caused by pneumococci and even more specifically where the mammal is a human.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A61K 35/84 // A61P31/00, A61P37/08, A61P33/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNAL, WPI DATA, PAJ, BIOSIS, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Week 200374 Derwent Publications Ltd., London, GB; Class D13, AN 2003-785685 & KR 2003021096 A (HUR T W ET AL), 12 March 2003 (2003-03-12) abstract	1-2,6-10
A	--	3-4
X	OSAKI YOSHIKO ET AL, "Antimutagenic and Bactericidal Substances in the Fruit Body of a Basidiomycete Agaricus blazei Agaricus blazei", Yakugaku Zasshi, Jun 17, 1994, Vol. 114, No. 5, p. 342-350, see abstract, fig 5	1-2,6-10
A	--	3-4

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
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Date of the actual completion of the international search	Date of mailing of the international search report
12 Sept 2005	15 -09- 2005
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STN International, File CAPLUS, CAPLUS accession no. 2001:461386, document no. 136:4949, Zhugiu, Ye et al: "Antibacterial activity of Agricus blazei", & Shipin Kexue (Beijing) (2001), 22(4), 82-84	1-2,6-10
A		3-4
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X	SORIMACHI KENJI ET AL, "Inhibition by Agaricus blazei Murill Fractions of Cytopathic Effects Induced by Western Equine Encephalitis (WEE) Virus on VERO Cells in Vitro", Biosci. Biotechnol. Biochem. 2001, Vol. 65, No. 7, p. 1645-1647	1-2,6-10
	--	
A	WO 2003020944 A2 (MEDIMUSH APS), 13 March 2003 (13.03.2003), page 5, line 10; page 15, line 4	1-4,6-10
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A	DATABASE WPI Week 200350 Derwent Publications Ltd., London, GB; Class B04, AN 2003-527661 & JP 2003040785 A (YASUKAI KK ET AL), 13 February 2003 (2003-02-13) abstract	1-2,3-4,6-10
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A	CHEN L. ET AL, "Coimmunization of Agaricus blazei Murill extract with hepatitis B virus core protein through DNA vaccine enhances cellular and humoral immune responses", International Immunopharmacology March 2004, Vol. 4, No. 3, p. 403-409	1-4,6-10
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X	EP 0413053 A1 (NIPPON HYPOX LABORATORIES INCORPORATED), 20 February 1991 (20.02.1991), see page 5, line 34-35, claim 1, claim 8	1,5-6,10
A		7-9

INTERNATIONAL SEARCH REPORT

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 0185191 A1 (TSUKUBA BIOSYSTEM, LTD.), 15 November 2001 (15.11.2001)	1,5-6,10
A	--	7-9
X	DATABASE WPI Week 200006 Derwent Publications Ltd., London, GB; Class B04, AN 2000-065421 & JP 11 279204 A (SUMITOMO FORESTRY CO LTD), 12 October 1999 (1999-10-12) abstract	1,5-6,10
A	--	7-9
A	DATABASE WPI Week 199952 Derwent Publications Ltd., London, GB; Class B04, AN 1999-604899 & JP 11263732 A (ICHIMARU PHARCOS INC), 28 September 1999 (1999-09-28) abstract	1,5-10
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A	DATABASE WPI Week 100017 Derwent Publications Ltd., London, GB; Class D13, AN 2000-189118 & JP 20 00032946 A (KYODO KENKO SHIZEN SHOKUHIN KK), 2 February 2000 (2000-02-02) abstract	1,5-10
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO2005/000012

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This International Search Authority considers there are two inventions covered by the claims indicated as follows:

I: Claims 1 (partly), claims 2-4, claims 6-10 (partly) directed to the use of Agaricus blazei Murill for producing a medication for combating or preventing bacterial and non-bacterial infections.

.../...

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

.../... continuation of box III

II: Claims 1(partly), claim 5, claims 6-10 (partly) directed to the use of Agaricus blazei Murill for producing a medication for combating or preventing allergies.

The present application has been considered to contain two inventions which are not linked such that they form a single general inventive concept, as required by Rule 13 PCT for the following reasons:

Invention I 1 relates to the problem of treating infections.This problem appears to be solved by using Agaricus blazei Murill for producing a medication for treating infections.

Invention II relates to the problem of treating allergies.This problem is solved by using Agaricus blazei Murill for producing a medication for treating allergies.

As the diseases are so technically different, no single general concept can be formulated based on the technical features of the inventions. Consequently, the requirements of Rule 13.1 PCT are not met.

INTERNATIONAL SEARCH REPORT

Information on patent family members

28/05/2005

International application No.
PCT/NO 2005/000012

WO	2003020944	A2	13/03/2003	NONE		
EP	0413053	A1	20/02/1991	JP	1161801 U	10/11/1989
				JP	1228480 A	12/09/1989
WO	0185191	A1	15/11/2001	AU	4455201 A	20/11/2001
				EP	1280544 A	05/02/2003
				JP	2004501096 T	15/01/2004
				US	20030104006 A	05/06/2003

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USE OF THE MUSHROOM *AGARICUS BLAZEI MURILL* FOR THE PRODUCTION OF MEDICAMENTS SUITABLE FOR TREATING INFECTIONS AND ALLERGIES

Area of the invention

The present invention concerns the use of the mushroom *Agaricus blazei Murill* (AbM) for producing a medication for treating or preventing bacterial and non-bacterial infections (e.g. parasites or virus) in mammals as well as treating or preventing allergy in mammals. Such an infection may e.g. be caused by pneumococci and even more specifically where the mammal is a human.

Introduction

Use of medical mushrooms has been a part of traditional Asian culture for more than 3000 years.

Many substances from mushrooms have been proven to affect the immune system and to be useable for treating a number of diseases (Wasser et al., 1999). In Japan there has been performed much research on the health effects of mushrooms (Ikekawa 2001). *Agaricus blazei Murill* (AbM) from the family *Basidomycetes* is such a medicinal mushroom that is very popular in Japan and is cultured artificially (Chen 2000) for the health diet market. This mushroom grows naturally near a small Brazilian village, Pietade, outside of São Paulo where id is daily large climatic changes. In this area where AbM was used in the food, the local population seemed to have a low incidence of cancer and other health problems (Huang 1997). In 1965 Dr. Takatoshi Furumoto sent AbM-spores to Japan and scientists at the National Cancer Center Research Institute of Japan, and supported by the Japanese Pharmacological Society, published in time results that proved that AbM had cancer-reducing properties. AbM is rich in immunostimulating and cancer-counteracting sugar molecules (polysaccharides) such

as beta (1,3) and (1,6) glucans (Kawagishi et al., 1989; Iwade & Mizuno, 1997; Huang 1997; Stamets 2000; Ohno et al., 2001; Sorimachu et al., 2001).

Extracts from the edible mushroom *Agaricus blazei Murill* (AbM) has been used for the last 10-20 years in Japan as a health diet against a number of diseases such as cancer, diabetes, arteriosclerosis and chronic hepatitis.

All of these diseases are, however, caused by weakening/abnormalities in cells in the suffering person, and do not have its origin in attacks from external organisms such as bacteria.

The cancer-inhibiting effect of AbM-components is scientifically documented in mouse models and on cancer cells (Itoh et al., 1994; Fujimiya et al., 1998; Ebina & Fujimiya, 1998; Takaku et al., 2001; Menoli et al., 2001; Bellini et al., 2003). AbM mycelium has also been proven to inhibit destroying effects (cytopatic) of WEE (Western Equine Encephalitis) virus on cells in culture (Sorimachi et al., 2001). NB - this article did not investigate the effect of AbM mycelium on the viral infection per se. Else there are not available English-texted reports in public databases that document other health effects of AbM, and not towards infections either.

General disclosure of the invention

The edible mushroom *Agaricus blazei Murill* (AbM) which grows naturally outside of São Paulo, Brazil, has for the last 10 years been cultivated artificially and has been used in heath food products in Japan to protect against a number of the diseases mentioned supra, including cancer. Even if such a use of this mushroom is known, it is not obvious that the mushroom also should be active against bacterial infections. Many health food products are considered to be acting curatively or preventively on

diseases without this having been documented. Furthermore, it is not immediately obvious that even if a product is known to enhance the immune system, the same product would be active against bacterial infections. Neither is it obvious that the effect of β-glucans generally would indicate that extracts from the fungus *Agaricus blazei Murill* would be active against bacterial infections, nor that AbM actually is more active than other natural medications in this field.

The effect of extracts of AbM against bacterial infection in mice according to the present invention has been investigated in a model wherein the mice are exposed to a mortal infection of pneumococci (*Streptococcus pneumoniae* serotype 6B). The AbM-extract was given via gavage to the mice from 24 hours to immediately prior to the injection of pneumococci into the peritoneal cavity. There were taken blood samples daily for bacterial cultivation from a femoral vein of the mice and the survival rate of the mice was registered. It was found that a dose of AbM-extract given with gavage either 24, 2 or 0 hours prior to the bacterial challenge, reduced the bacterial count in the blood and increased to survival rate of the animals with respect to animals having been given saline via gavage. As much as 50% of the animals that were given an AbM extract 24 hours prior to challenge survived at day 10 versus 13% of the control animals at day 7. This proves that the extract from AbM may be used for protection against and optionally as a treatment for pneumococcal infection.

In times with increasing antibiotic resistance AbM may be a natural alternative or supplement to antibiotics and optionally other anti-infection substances, but with fewer detrimental side effects as well as the positive side effect as a cancer-protective substance.

The pneumococcus *Streptococcus pneumoniae* is a gram-positive diplococcus causing potentially lethal diseases

such as blood poisoning (sepsis) and brain membrane inflammation (meningitis), but also infections of lesser seriousness such as lung, middle ear and sinus cavity inflammation. There exist 90 subgroups (serotypes) of pneumococci, inter alia serotype 6B (Henrichsen 1979) which has a moderate infectious effect (virulence) and consequently gives a relatively prolonged, but still lethal, progression of the disease in mice) Aaberge et al., 1995). Since the frequency of antibiotic-resistant bacteria, e.g. multiresistant *S. pneumoniae*, is a hazard for the public health and antibiotics in a few decades probably has a reduced or lacking effect, it should be attempted to find good alternative preventive and treating principles.

β-glucans are known immunomodulating substances (Riggi & DiLuzio, 1961; Boegwald et al., 1984) and are main components of the cell wall in fungi any yeasts. β-glucans have anti-infection (Reynmolds et al., 1980; Franek et al., 1992) and anti-cancer (Tagucho et al., 1983; Ohno et al., 1987) effects in animal models. A 1,3-β-glucan in the fruit body in AbM may be the anti-cancer principle of the fungus (Ohno et al., 2001).

Previously it has been found that β-glucans (inter alia SSG from the fungus *Sclerotinia sclerotorium* and from yeast), as well as a sugar molecule from common plantain, *Plantago major L.*, protects against infection with BCG and pneumococci in mouse models (Hetland et al., 1998; Hetland et al., 2000a, b; Hetland, 2003). These effects were observed after injection of the substances in the abdominal cavity (intraperitoneal injection) of the mice, but were not confirmed after gavage feeding. Tests proved that the protective effect was due to stimulation of the hereditary immune system where the macrophage is a central immune cell. It has also been proven that SSG and MacroGard® from yeast inhibits the growth of the tubercle bacterium,

Mycobacterium tuberculosis, in macrophage cell cultures (Hetland & Sanven, 2002).

One of the aspects behind the present invention is to use an AbM-extract for producing a medication that protects against bacterial infections exemplified by the lethal pneumococcal infection in mice with the serotype 6B. This was done by supplying the pneumococci to the mice through the aid of a gavage. The effect of the AbM extract was evaluated based on bacterial count in venous blood and the survival rate of the animals.

It is a further aspect of the present invention to use an extract from the mushroom *Agaricus blazei Murill* to produce a medication that combats or softens allergy in mammals, especially humans.

Allergy is an ever increasing problem in the western world, among them Norway. Extracts from the mushroom *Agaricus blazei Murill* (AbM) is traditionally used, as mentioned supra, in Japan against several diseases, among others cancer, and the effect of AbM against a type of cancer is documented. AbM contains immune-stimulating polysaccharides such as β-glucans, and these have been proven previously to work immunomodulatingly and to provide the mentioned protection.

As a background for the surprising discovery concerning extracts from the mushroom *Agaricus blazei Murill*, the following circumstances will be summarized briefly: The immune system is divided into the hereditary (which, without being bound by possible theories, AbM apparently affects) and the adaptive immune system. This is in turn divided into the T-helper cell-1, -2 and-3 responses (Th1, Th2 and Th3), wherein the Th1-response inter alia is important for the anti-infection and anti-tumour defence; Th2 for anti-parasite and anti-rejection defence, but promotes allergy; and Th3 provides anti-inflammation

(inflammation-suppression) and promotes the formation of new tissue. Additionally, there is now a strong focus on regulatory T-helper cells. According to the T-helper cell-1 (Th1/Th2-paradigm) these responses are inversely proportional because Th1 will inhibit Th2 and vice versa, so that a strong Th1-response is commensurable with a low Th2-response.

It has, as mentioned supra, been found that the AbM extract is effective towards infections exemplified through pneumococcal infection in a mouse model. However, there are indications pointing to the circumstance that there exist other substances in AbM that are equally important as glucans for the relevant anti-infection effect that has been found. Since the anti-infection effect is caused by a high Th1-response, it will, based on the mechanism of the immune system explained supra, be expected a simultaneously inhibited Th2-response. Since allergy is the result of a high Th2-response, the AbM-extract has surprisingly also a stimulating effect on the Th2-response, something which is surprising and unexpected based on the expected low Th2-response based on the protective effect that the AbM extract has against infections.

To investigate the effects that AbM has for inhibiting the development of allergy, the following test was done with a mouse model that was immunized with the model allergen ovalbumin (OVA). The level of IgE and IgG1 (Th2-allergic response) and IgG2a (anti-infection/cancer response) anti-OVA-antibodies was measured in the serum from the mice at the end of the test. The level of signal substances (cytokines) being secreted into the blood from stimulated immune cells was also investigated, something which will indicate the relevant Th1 (IFNγ, IL-12), Th2 (IL-5, IL-10, IL-13) or Th3 (TGFβ) response. Previously it has been shown production of the inflammation-increasing cytokines (TNF-α and IL-8) and NO^- (toxic nitrogen compound) from AbM-stimulated macrophages (white blood cells that are

important for the hereditary immune defence) (Sorimachi, 2001).

The relevant tests for supporting the anti-allergic effect of the AbM extract is given under the heading "Materials and Methods II" whereas the relevant tests for support of the anti-infection effect of the AbM extract is given under the heading "Materials and Methods I".

SUPPORT FOR THE ANTI-INFECTION EFFECT OF THE ABM-EXTRACT:

Materials and Methods I:

Mice.
All the animal tests were approved by the local representative for the national ethical committee for tests with animals, and were performed according to national standards from the Department of Agriculture. There were used inbred microbial-free female mice of the strain NIH/OlaHsd from Harlan Olac Ltd., England. The mice were 6 weeks old at arrival and rested for 1 week before the experiment.

Reagents
Extracts A, B, C, D and E from AbM mycelium were from different Japanese producers of health foods. Extract A ("gold label type") was the most purified product and extract B ("Katsu type") is a lesser purified product, both from ACE Co. Ltd., Gifku-ken, Japan. The producers of the AbM extracts C, D and E has not been informed about this study and the names will consequently not be disclosed. Phosphate buffered saline (PBS) was used as a control.

Bacteria
A strain of *Streptococcus pneumoniae* serotype 6B from RIVM, the Netherlands, was used. It was kept frozen and was used for contagion tests as known earlier (Aaberge et al., 1995).

Blood samples

It was taken blood samples from the external femoral vein on the hind legs (Saphena magna) of the mice. The blood was then cultivated as known previously (Aaberge et al., 1995).

Quantification of colony-forming units (CFU) in blood

Venous blood (25 µl) was diluted 10-fold in Todd-Hewitt agar, and 25 µl of diluted blood was distributed onto blood agar-plates which were incubated at 37°C in 5% CO_2. After 18 hours the colonies were counted.

Experimental procedure

Two experiments were performed with 7-9 animals in each treated group (Table 1, Figure legend). The volume of PBS or AbM-extract for gavage feeding was 200 µl. All the animals were bled at the times indicated in the figures, and the blood was distributed onto agar plates. The animals were inspected daily and mice that were very ill were sacrificed by neck stretching.

Measurements

This was bacterial content in peripheral blood determined by *S. pneumoniae* CFU count, and the survival rate of the animals.

Statistics

Parametric tests were used on normally distributed data, else non-parametrical tests. One-way repeated measurements ANOVA/Turkey's test was used for multiple comparisons, and paired t-test for single comparisons. P-values below 0,05 were considered to be statistically significant.

Results

Effect of AbM-extract given 2 hour prior to challenge on S. pneumoniae serotype 6B infection

Mice were given PBS or one of the 5 AbM-extracts (A-E) from different producers via gavage 2 hours before injection into the abdominal cavity (i.p.) of *S. pneumoniae* serotype 6B. Blood samples for bacterial cultivation were taken daily from the femoral vein and the illness of the animals was surveyed. Only AbM-extract A gave a significantly reduced CFU-level as compared to the PBS control ($p<0,05$) (Fig. 1). The survival rate of mice given AbM-extract A was also higher than for mice given PBS ($p<0,05$) (Fig. 2). Even if no control animals survived day 5 after contagion, 38% of the animals in group A were alive after 6 days. Among these 25% were still alive on day 7, but had to be sacrificed on account of neurological complications. The AbM-extract D showed a tendency to lower bacterial counts in blood and increased survival, but the differences were not statistically significant in relation to PBS (Figs. 1, 2).

Effect of AbM-extract given 24 hours prior to or with contagion on S. pneumoniae 6B infection.

In the next experiment AbM-extract or PBS was given either 24 hours, 2 hours or immediately prior to contagion. Even if the finding supra with AbM-extract A given 2 hours prior to contagion was not statistically significant, experiment 2 showed the same tendency (Figs. 3, 4). The preventive positive effect of AbM-extract A was statistically confirmed when the extract was given 24 hours prior to contagion, both with respect to bacterial count in blood ($p<0,05$) (Fig. 3) and survival rate ($p<0,05$) (Fig. 4). There were also similar and significant results when extract A was given prior to contagion. Actually, 38% of the animals survived that received AbM-extract A two or 0 hours prior to contagion day 10 in this test as compared to 10-20% of the controls after day 7. The best result was obtained when extract A was given 24 hours prior to contagion since this gave a survival rate after 10 days of

all of 50% (Fig. 4) as compared to PBS control of 13% after 7 days.

Discussion

In contrast with previous experiments with β-glucans and a sugar extract from the wound-healing plant *Plantago major L.* (common plantain) given i.p. in the disclosed infection model in mice, the AbM-extract was equally effective when it was given with gavage. The β-glucan with the highest effect after i.p. administration did not have any effect when it was given via gavage to the mice in this pneumococcus infection model. This makes the AbM-extract probably more useful than β-glucan because it does not require sterilization of the product for intravenous injection and thus strict GMP (good manufacturing practice) requirements, and that the product also may be ingested outside of a hospital. We have previously shown that the β-glucans SSG and MacroGard® also strengthen the establishment of allergies in a mouse model (Ormstad et al., 2000; Hetland et al., 2000). The AbM-extract A given via gavage in the same model does not show any such side effect. Quite the contrary, the results with the allergy model indicate that the AbM-extract protects against the development of allergies.

The graphs for bacterial content I blood climbed more steeply in test 1 than 2 on account of the injection of the double number of *S. pneumoniae* CFU in the first ($1,92 \times 10^6$ CFU) as compared to the second ($0,97 \times 10^6$ CFU) experiment. The purpose was to challenge the animals with $100 \times LD_{50}$ (lethal dose for 50% of the individuals) ($= 100 \times 1,2 \times 10^4$ CFU (Aaberge et al., 1995)) for *S. pneumoniae* serotype 6B. However, because the number of CFU given is calculated from the number of bacterial CFU that was frozen after the previous cultivation, the exact number of live bacteria, i.e. CFU, that is injected will not be known before the answer form the cultivation of a parallel bacterial sample

is present. The lower number of bacteria that was injected in experiment 2 also gave a higher survival rate (10-20% after 7 days) of the control animals as compared to experiment 1 (0% after 3 days). This is probably the reason for the lacking statistically significant difference between AbM-extract A and PBS given 2 hours prior to contagion.

The effect of AbM-extract A given at the same time as contagion also points towards a possible positive treatment effect of the extract. This was not given afterwards on account of early high mortality of the test animals in the control group in this infection model. This will be tested in another infection model with lower mortality. Since the immune system uses similar mechanisms to combat cancer cells and virus-infected cells, namely natural killer (NK) cells and cytotoxic T-lymphocytes, and since AbM is effective towards cancer, AbM will probably also have a positive effect towards viral infections.

AbM may probably be used as a supplement to vaccines in exposed groups, e.g. persons that have had their spleen removed and who thereby, as known, is more prone to get pneumococcal pneumonia and blood poisoning. Other relevant target groups may be tourists who are to travel to countries with poor hygiene or surgical patients to whom it is given as preventive antibiotic prophylaxis prior to an operation. It is also conceivable that a more general use of a "immune stimulating" substance such as AbM may decrease the use of antibiotics and "over-vaccination" and give the immune system a better opportunity to "adapt" to fighting microbes, and thus also have a reducing effect on the development of allergies. According to the hypothesis of hygiene the increased allergy frequency in western countries is caused by the population being more protected against disease-causing microbes. The fact that AbM has been proven to protect against cancer in a mouse model, and that there are no known side effects of the AbM extract in

millions of Japanese users of health products, also increases the use value of AbM as a prophylactic/therapeutic substance.

Conclusion

The present results show that an AbM extract protects against deadly pneumococcal infection in mice when the extract is given via gavage. Only highly purified extracts ("gold label") have a significant effect. A positive effect was found when the extract was given from 24 hours prior to until immediately prior to bacterial contagion. This was demonstrated through the use of lower bacterial count in blood and increased survival in animals that received AbM extract as compared to animals that received saline. The fact that the extract is active after ingestion through the digestive system, makes AbM very interesting as an antibacterial medicinal substance. The AbM extract may act prophylactic towards, and probably also act therapeutically towards an infection involving especially bacteria, but probably also other disease-mediating microorganisms. In a time with increasing resistance towards antibiotics, AbM will be a natural supplement or an alternative, with fewer side effects, to antibiotics and optionally other anti-infection substances as well as having a positive side effect as a cancer-inhibiting substance.

The tables and figures indicated infra relate to the tests that have been disclosed supra.

Table 1

Test protocol for AbM-treatment via gavage of NIH/OlaHsd mice infected with pneumococci (*Streptococcus pneumoniae*) of serotype 6B.

A) Experiment 1: Treatment with different AbM extracts 2 hours prior to contagion.

Group	Day 0,-2h	Day 0,0h	Day 10
AbM A	Extract A	Pn6B $1,9x10^6$ CFU	End
AbM B	Extract B	Pn6B $1,9x10^6$ CFU	End
AbM C	Extract C	Pn6B $1,9x10^6$ CFU	End
AbM D	Extract D	Pn6B $1,9x10^6$ CFU	End
AbM E	Extract E	Pn6B $1,9x10^6$ CFU	End
PBS	PBS	Pn6B $1,9x10^6$ CFU	End

B) Experiment 2: Treatment with AbM A extract at different times prior to contagion

Group	Day -1	Day 0,-2h	Day 0,0h	Day 0,0h	Day 10
AbM -24h	Extract A			Pn6B $x10^6$ CFU	End
PBS -24h	PBS			Pn6B $x10^6$ CFU	End
AbM -2h		Extract A		Pn6B $x10^6$ CFU	End
PBS -2h		PBS		Pn6B $x10^6$ CFU	End
AbM 0h			Extract A	Pn6B $x10^6$ CFU	End
PBS 0h			PBS	Pn6B $x10^6$ CFU	End

Abbreviations: AbM (*Agaricus blazei Murill*), Pn (Pneumococci).

Figure legend

Fig. 1.

Number of pneumococci of serotype 6B CFU I peripheral blood from NIH/Ola Hsd female mice pre-treated with AbM extract A-E or PBS via gavage (volume 200 µl) 2 hours prior to injection in the abdominal cavity (i.p.) with 1,92 x 10^6 CFU of pneumococci type 6B (see Table 1). The animals were exsanguinated at the specified intervals, the samples distributed and the number of CFU counted. Dead animals are specified as animals with 1 x 10^9 CFU in the blood. The data points represent median values from 8 animals and show lower CFU-levels in AbM extract A-treated animals.

Fig. 2.

Survival rate (median values) for the mice in Fig. 1 that were pre-treated with AbM extracts or PBS 2 hours prior to i.p. contagion with pneumococci serotype 6B. The data points represent median values from 8 animals and show a higher survival of AbM extract A-treated animals.

Fig. 3.

Number of pneumococci of serotype 6B CFU I peripheral blood from NIH/Ola Hsd female mice pre-treated with AbM extract A or PBS via gavage (volume 200 µl) 24 or 2 hours or immediately prior to (i.p.) injection with 0,97 X 10^6 CFU of pneumococci type 6B (see Table 1). The animals were exsanguinated at the indicated intervals, the samples were distributed and the number of CFU was counted. Dead animals are indicated as animals with 1 x 10^9 CFU in the blood. The data points represent median values from 8

animals and show lower CFU-levels in AbM extract A-treated animals. Note: logarithmic scale on the Y-axis.

Fig. 4.

Survival rate (median values) for the mice in Fig. 3 which were pre-treated with AbM extract A or PBS 24-0 hours prior to i.p. contagion with pneumococci serotype 6B. The data points represent median values from 8 animals and show higher survival especially for animals treated with AbM extract A 24 hours prior to contagion.

Fig. 5.

Effect of AbM p.o. on IgE anti-OVA-levels in OVA-immunized mice.

Fig. 6.

Effect of AbM p.o. on Ig2a anti-OVA-levels in OVA-immunized mice.

Fig. 7.

Effect of AbM on faecal bowel-membrane inflammation (peritonitis) in Balb/c-mice that received AbM p.o. on day -1 and 1/8 faeces-dilution i.p. on day 0. The figure shows survival (Kaplan-Meier-plot).

Fig. 8.

THP-1-cells stimulated with AbM and endotoxin.

Fig. 9.

The figure shows a "scatter-plot" - F365 Mean - B635 vs. F532 Mean - B532 microarray of genes that are upregulated

against genes that are downregulated under the influence of the extract from *Agaricus blazei Murill*.

Fig 10.

The figure shows specific IgE levels in NIH/Ola-mice sensitized with ovalbumin (OVA) and then treated p.o. with *Agaricus blazei Murill* (AbM) or PBS before OVA booster.

According to the present invention it is preferred to give the AbM extract with the antibacterial effect in combination with at least one further medicinal substance where it furthermore is preferred that the additional medicinal substance is an antibacterial substance.

It is also further preferred to give the present AbM extract as an oral preparation. In this connection the extract may be given per se, but it may also be combined with common carriers and exipients so that it may be given as a liquid substance e.g. an elixir, a mixture, a tincture etc. Alternatively the AbM extract may be given in the form of a solid medication such as a pill, a tablet, a capsule, a lozenge etc. In this connection the medication may also be provided with usual additives such as taste additives (sugars, sweeteners etc.) and colorants.

For further supporting the anti-infection effect of extracts of AbM it was established that an extract from AbM had protective effect also against bowel-membrane-inflammation (peritonitis) in Balb/c-mice infected i.p. with a faeces-dilution. The AbM extract was given with gavage p.o. 24 hours prior to inoculation i.p. and temperature (measured by the aid of scanning a temperature chip implanted in the neck skin of the mice), bacteraemia in peripheral blood and survival was investigated. There were significant differences in all these parameters versus control mice that were treated with physiological saline

p.o. instead of AbM. Fig. 7 shows the positive effect of AbM on the survival of faeces-infected mice.

Monocytes in blood and monocyte-derived macrophages in the tissues are central immune cells in the hereditary immune system that active components in *Agaricus* affect. To study the stimulating effect of *Agaricus* on such cells there was used the human promonocyte cell line THP-1 which was cultivated for 24 hours in the presence or absence of 10% sterile filtrated AbM extract. There were investigated both the secretion of signal substances (cytokines) from the cells to the cell culture supernatant and up- or downregulating of genes that code for cytokines. Secreted cytokines was determined through the aid of ELISA-methods, and the results show that *Agaricus*-stimulation of the cells increased the secretion of central inflammation-enhancing (pro-inflammatory) cytokines interleukin (IL)-6 and IL-8 (inter alia chemo-attractants for T-lymphocytes and neutrophile granulocytes), whereas the excretion of a central inflammation-reducing (T-cell regulatory) cytokine such as TGFβ was reduced (Fig. 8). A similar effect on IL6 was also proven in primary monocytes from peripheral blood (not shown). On the other hand there was no secretion of IL-4 (allergy-promoting) or IL-10 (inflammation-reducing/slow) cytokines from the cells.

Most importantly are, however, the findings done by the aid of micro-array-technique where mRNA (genetic signal material for single genes) isolated from cells that have been stimulated or not stimulated with a substance, compete for binding to a probe on a chip whereon the complementary nucleotide bases for mRNA to the genes that are to be investigated, are printed. Where the substance stimulates expression of a certain gene there will be made more mRNA molecules that displaces (out-competes) the binding to the probe of mRNA for this gene from non-stimulated cells. mRNA from stimulated cells and controls are labelled with red and green fluorescent colour that is used when reading

the result of the binding by the aid of an instrument that quantifies light signals with a wavelength for the relevant red and green light. Micro-array of THP-1-cells stimulated with AbM extract for 24 hours did show a strongly increased upregulation of genes for pro-inflammatory cytokines such as IL-1, IL-8 and TNFα, as well as the newly discovered genes for enhancing the anti-infection and anti-tumour defence (Th1 cytokine), i.e. IL-23α subunit p19 that is included in (Th1 cytokine family) the IL-12-family. On the other hand the gene for IL-4 or IL-10 was not upregulated. Fig. 9 shows such a microarray after competition for binding between gene products from the control cells and cells stimulated with AbM extract.

The results of these cell tests show that the AbM extract stimulates the anti-infection defence (increased Th1-response) and does not increase a central allergy-inducing cytokine such as IL-4 (gives a Th2-response). When the literature now states that there is a balance between the Th1- and Th2-responses such that an increase in one leads to a decrease in the other, this indicates that an increased Th1-response gives a decreased Th2-response as is observed in the results from the allergy mouse model (see infra). The fact that the anti-infection defence is stimulated by the AbM extract means that the body's defence towards infections per se is strengthened, it be bacteria, virus or parasites. Consequently the effect that has been shown from the AbM-extract towards infections (bacterial and non-bacterial) and allergies, combined with the knowledge that exists concerning immunological principles, will verify that the AbM-extract will have such a general effect, as is claimed in the present patent claims.

TESTS FOR SUPPORTING THE ANTI-ALLERGIC EFFECT OF THE ABM-EXTRACT:

In connection with the allergy-protecting effect of extracts from *Agaricus blazei Murill* the following test were performed:

Materials and Methods II

Mice: Balb/c females, 6 weeks old at arrival and rested for 1 week in animal stables.

Reagents: Enzyme-fermented extract A ("gold label") of AbM-mycelium from ACE Co. Ltd., Japan, PBS and OVA.

Blood sampling: The animals were drained at ended experiment in CO_2-anaesthesia and serum was frozen at -20°C.

Experimental procedure: The mice (n=8/group) were fed by gavage with 200 µl AbM-extract or PBS on day -1. The mice were then immunized s.c. in their tail root with OVA + $Al_2(OH)_3$ (adjuvant) on day 0 and again on day 20 (booster dose for increased allergy response). The experiment was ended after 26 days, when the IgE and anti-OVA response is peaking in this model, after the first OVA immunisation with heart puncture and draining (for serum) of the animals under CO_2-anaesthesia. Serum from the animals was analysed for IgE, IgG1 and IgG2a anti-OVA and level of cytokines, and drained on day 26.

Measurements: The levels of IgE, IgG1 and IgG2a antibodies in serum against OVA were measured by using ELISA-technique. The level of cytokines (IFNγ, IL-5, IL-10, IL-12, IL-13, TGFβ) that are typical for TH1-, Th2- and Th3-responses, were measured in serum and supernatant from cultivated abdominal macrophages and spleen cells from the animals. Measurements of cytokines were not performed.

Statistical evaluation of the results was performed as explained supra.

From the experiments it was found a decreased (p=0,17) IgE anti-OVA-level in serum from mice that had received AbM per os versus the ones that had received PBS (Fig. 5). This shows that AbM inhibits the development of allergy against OVA on account of the inhibited Th2-response. Additionally the results show that the IgG2a anti-OVA-level was higher in the group that had received AbM versus the control (PBS-group) (Figure 6). This shows that AbM gives an increased Th1-response, something that fits the decreased Th2-response shown by the IgE-analysis. IgG1 displayed increased reverse levels. Admittedly, this was not supported by the IgG1 anti-OVA-measurements, but this test is under development and is still not one hundred percent reliable so that this finding is not considered to be significant. As opposed to this there has previously been found that β-glucans such as scleroglycan (Ormestad et al., submitted) (given i.p.) boosts the development of allergy in this relevant mouse model. This proves that there exist other factors than β-glucan in the AbM-extract that are effective in the animals, and this forms a basis for the object of the present invention since it would have been assumed by the person skilled in the art that substances promoting a given immune response would not be active in an opposite immune response (see supra concerning the effects of Th1, Th2 and Th3).

Therapeutic anti-allergic effect of AbM

NIH/Ola mice were immunized s.c. with ovalbumin (OVA) and treated with AbM extract or PBS (200 µl each) via a gastric catheter 20 days later and a day before OVA booster. The animals, 8 in each treatment group, were sacrificed and exsanguinated 5 days later, and serum IgE (Th2 response) or IgG2a (Th1 response) anti-OVA antibodies measured by ELISA. Two such experiments were run. It was found that the levels of IgE anti-OVA were significantly (p=0,04) lower AbM-treated mice relative to the PBS-treated once (Fig 10). On the other hand, there was no difference in levels of

IgG2a anti-OVA between the groups (data not shown). This shows that the *Agaricus* mushroom, in addition to its preventive properties against allergy development shown above, also can be utilized as a therapeutic anti-allergic substance in individuals already sensitized to an allergen.

The invention concerns thus in a second aspect the use of the AbM-extract for producing medications that are suitable for preventing or combating allergies in mammals, especially humans. Among relevant allergic reactions that may be prevented/combated with compositions comprising the extract(s) from AbM according to the present invention there may be mentioned dust allergy (pollen allergy, hay fever, allergy against house dust etc.), food allergy (protein allergy e.g. fish allergy, milk allergy, shellfish-allergy etc.), contact allergy (allergy against animals such as dogs, cats, etc.).

C l a i m s

1. The use of *Agaricus blazei Murill* (AbM) for producing a medication for combating or preventing bacterial and non-bacterial infections and/or allergies in mammals.

2. The use according to claim 1, wherein the relevant non-bacterial infection is caused by a parasite or a virus.

3. The use according to claim 1, wherein the bacterial infection is caused by pneumococci.

4. The use according to claim 3, wherein the pneumococcus is *Pneumococcus pneumoniae.*

5. The use according to claim 1, wherein the allergy is selected from the group comprising dust allergy (pollen allergy, hay fewer, allergy against house dust, etc), food allergy (protein allergies, e.g. fish allergy, milk allergy, shellfish allergy, etc.), contact allergy (allergy against animals such as dogs, cats, etc.).

6. The use according to any of the claims 1 - 5, wherein the medication is an oral medication.

7. The use according to any of the claims 1 - 5, wherein the medication is an intravenous preparation.

8. The use according to any of the claims 1 - 7, wherein the medication comprises at least one further medicinal substance.

9. The use according to claim 8, wherein the further medicinal substance is an antibacterial substance.

10. The use according to any of the preceding claims, wherein the mammal is a human.

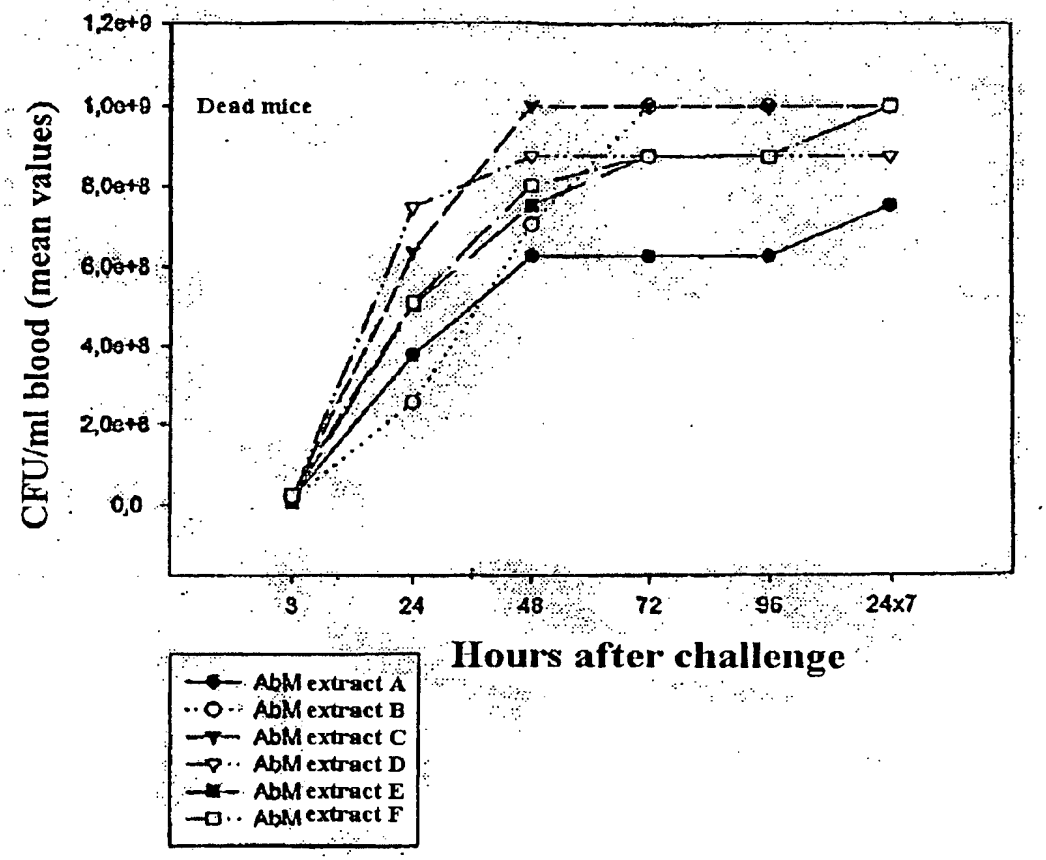

Dead mice
CFU/ml blood (mean values)
1,2e+9
1,0e+9
8,0e+8
6,0e+8
4,0e+8
2,0e+8
0,0
3
24
48
72
96
24x7
Hours after challenge
AbM extract A
AbM extract B
AbM extract C
AbM extract D
AbM extract E
AbM extract F

Fig.1

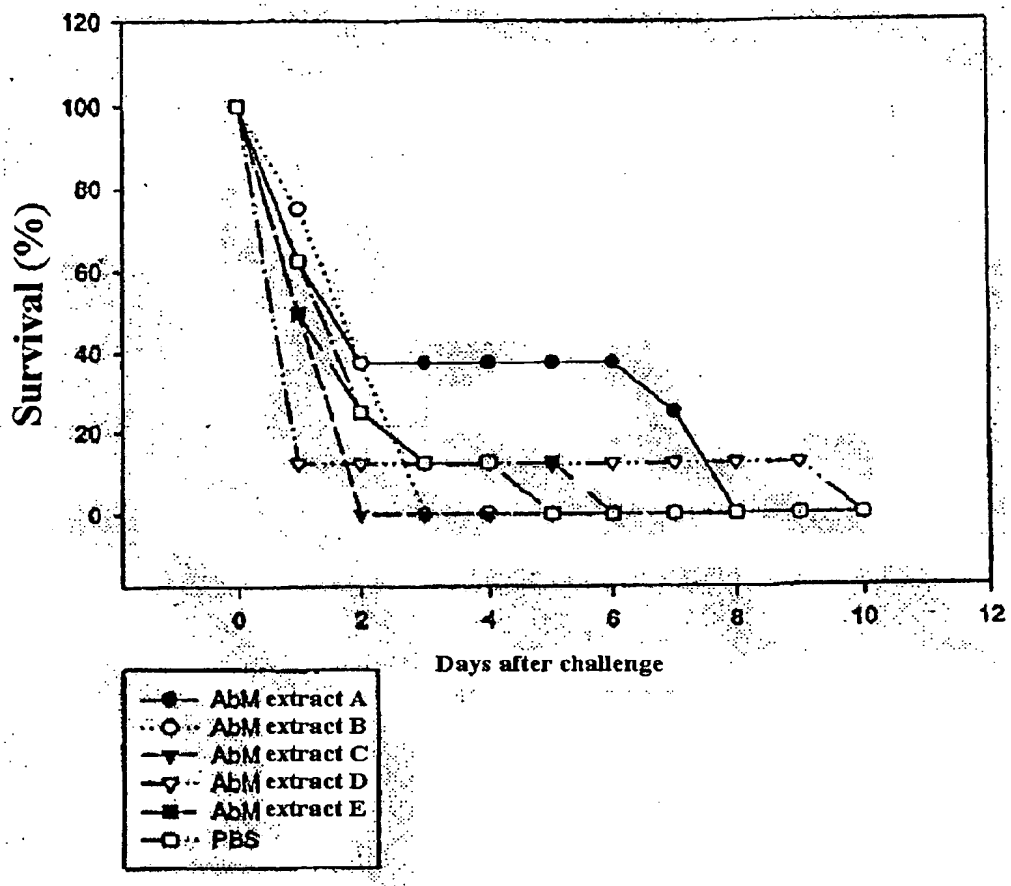

120
100
80
60
40
20
0
Survival (%)
0
2
4
6
8
10
12
Days after challenge
AbM extract A
AbM extract B
AbM extract C
AbM extract D
AbM extract E
PBS

Fig. 2

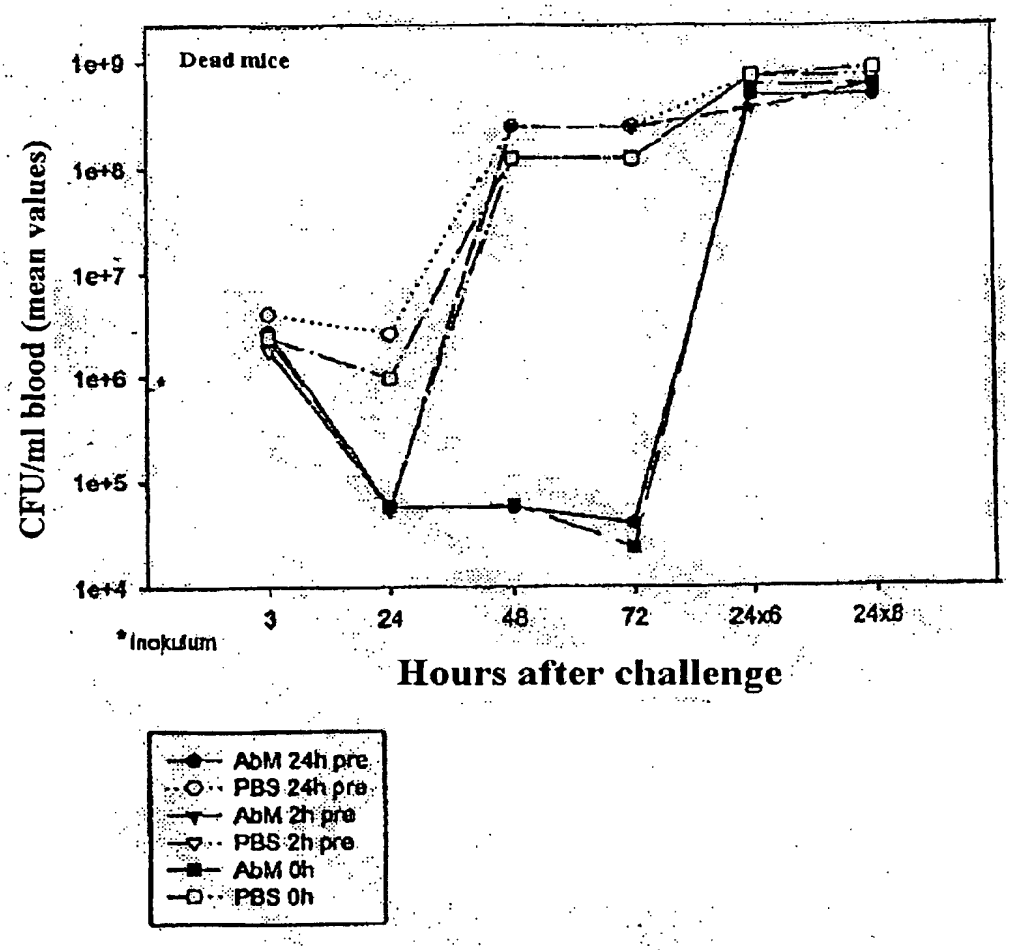

Dead mice
CFU/ml blood (mean values)
1e+9
1e+8
1e+7
1e+6
1e+5
1e+4
*Inokulum
3
24
48
72
24x6
24x8
Hours after challenge
AbM 24h pre
PBS 24h pre
AbM 2h pre
PBS 2h pre
AbM 0h
PBS 0h

Fig.3

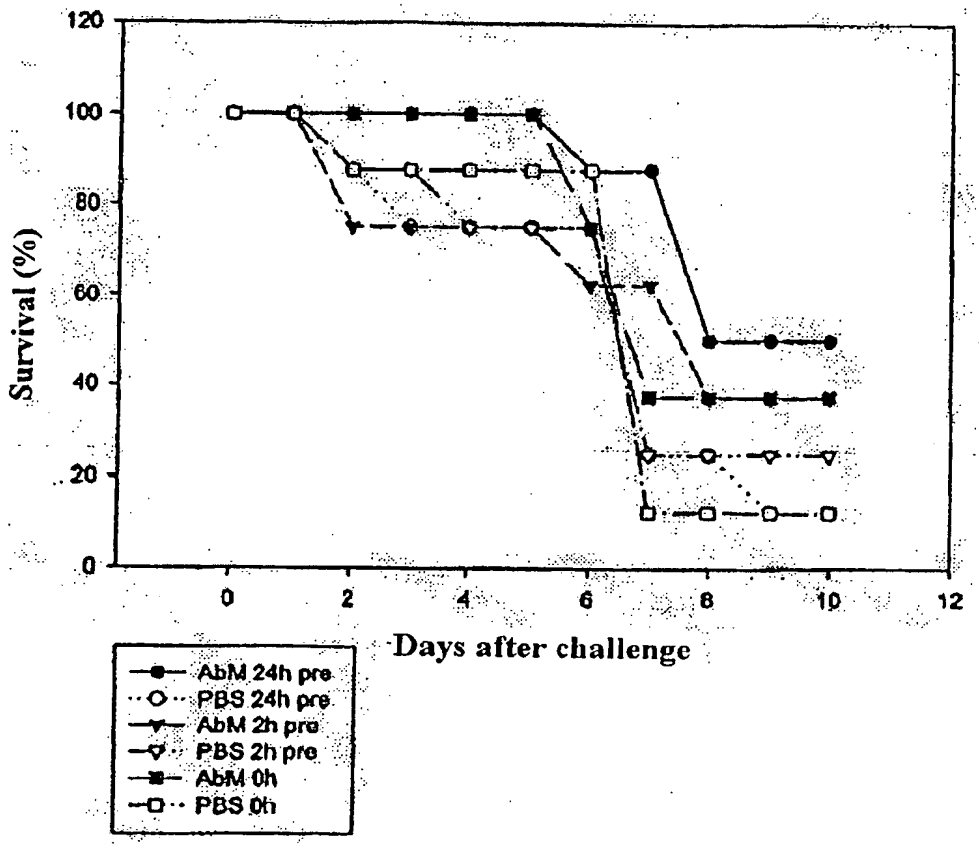
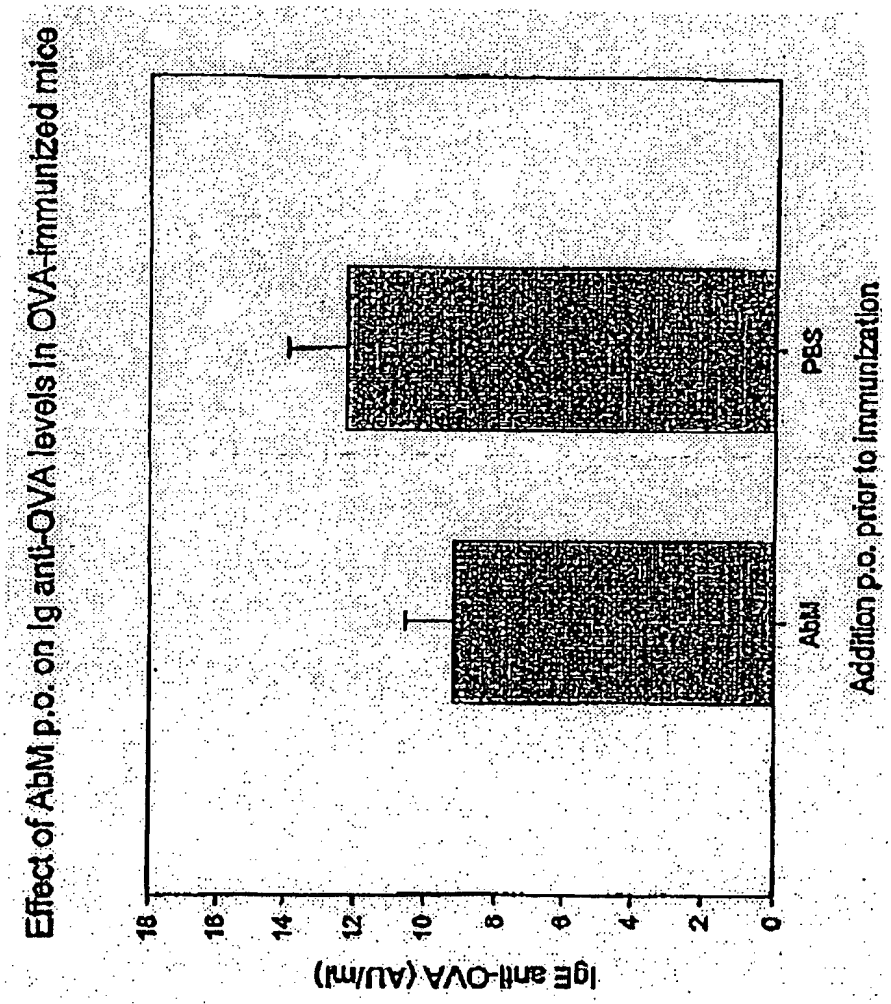

120
100
80
60
40
20
0
Survival (%)
0
2
4
6
8
10
12
Days after challenge
AbM 24h pre
PBS 24h pre
AbM 2h pre
PBS 2h pre
AbM 0h
PBS 0h

Fig.4


Effect of AbM p.o. on Ig anti-OVA levels in OVA-immunized mice
IgE anti-OVA (AU/ml)
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2
4
6
8
10
12
14
16
18
AbM
PBS
Addition p.o. prior to immunization

Fig.5

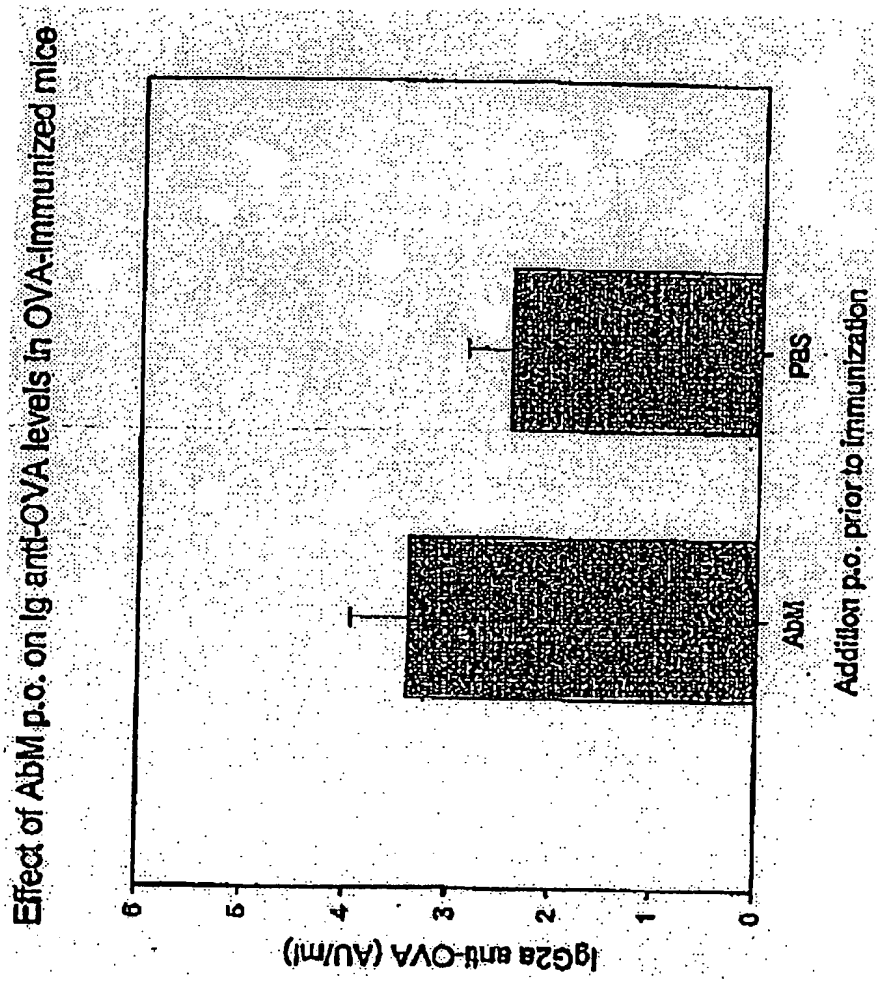

Effect of AbM p.o. on Ig anti-OVA levels in OVA-immunized mice
IgG2a anti-OVA (AU/ml)
0
1
2
3
4
5
6
AbM
PBS
Addition p.o. prior to immunization

Fig.6

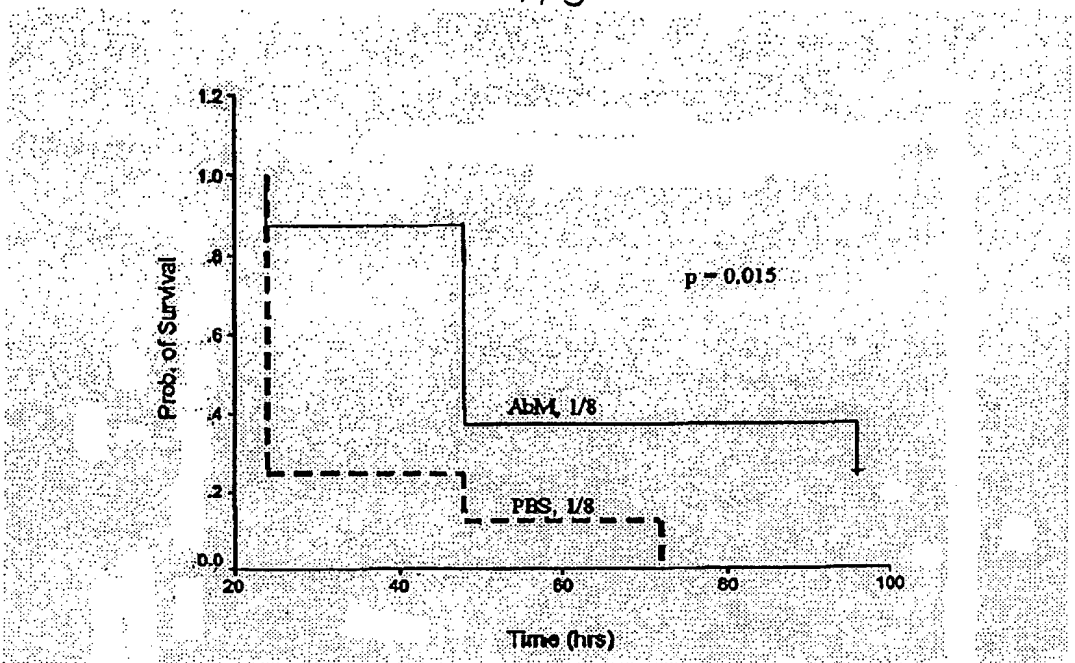

1.2
1.0
.8
.6
.4
.2
0.0
Prob. of Survival
p = 0.015
AbM, 1/8
PBS, 1/8
20
40
60
80
100
Time (hrs)

Fig.7

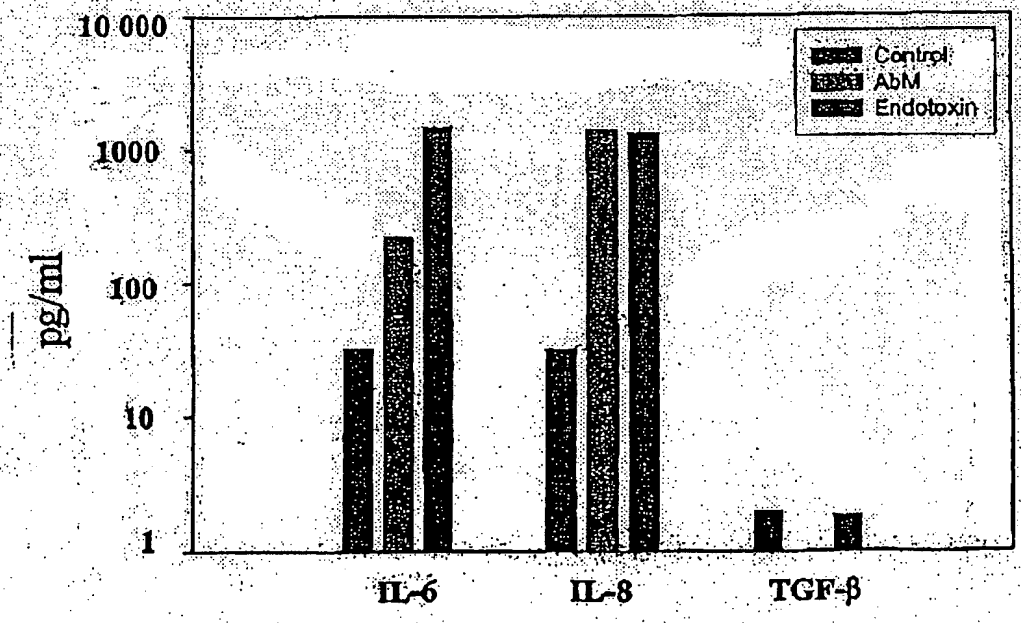

10 000
1000
100
10
1
pg/ml
Control
AbM
Endotoxin
IL-6
IL-8
TGF-β

Fig.8

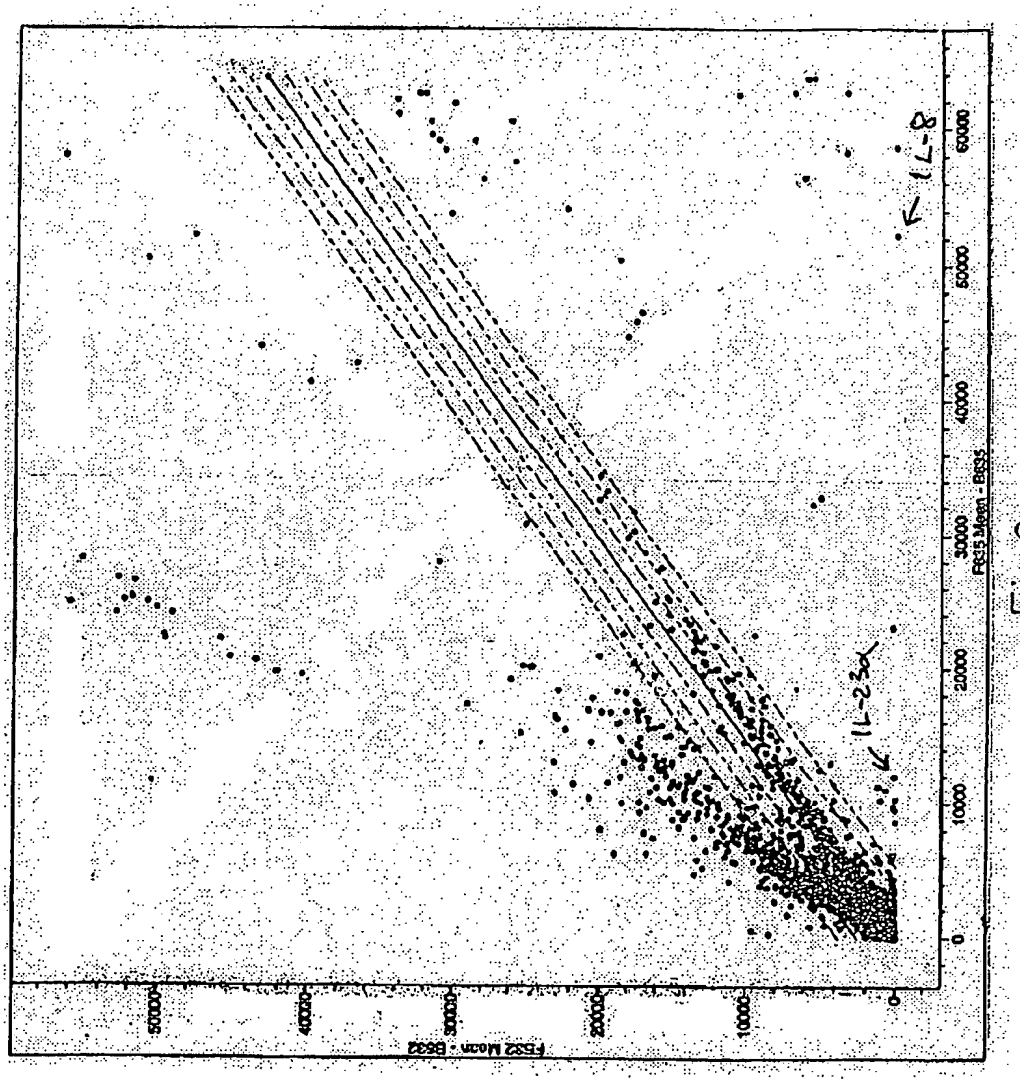
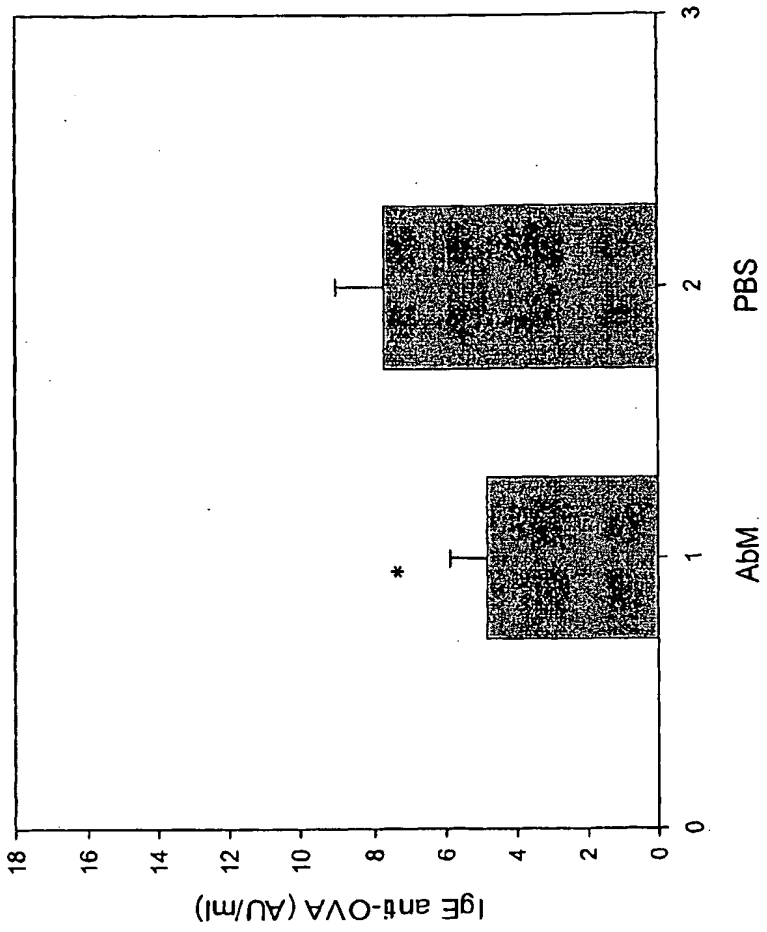

17-8
11-23
60000
50000
40000
30000
20000
10000
0
R635 Mean - B635
5000
4000
3000
2000
1000
0
F532 Mean - B532

Fig 9



*p=0.04

Fig.10

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference 159885 TG-KR	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/NO2005/000012	International filing date *(day/month/year)* 10 January 2005 (10.01.2005)	Priority date *(day/month/year)* 12 January 2004 (12.01.2004)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant HETLAND, Geir		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 17 July 2006 (17.07.2006)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Beate Giffo-Schmitt e-mail: pt03@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

REC'D 20 SEP 2005
WIPO PCT

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Oslo Patentkontor AS
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N-0206 Oslo
Norge

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	15 -09- 2005
Applicant's or agent's file reference 159885 TG-KR	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NO2005/000012	10.01.2005	12.01.2004

International Patent Classification (IPC) or both national classification and IPC
A61K 35/84 // A61P 31/00, A61P 37/08, A61P 33/00

Applicant
Geir Hetland

1. This opinion contains indications relating to the following items:

- [x] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [x] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [x] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further opinions, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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S-102 42 STOCKHOLM

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Authorized officer
Yvonne Siösteen/EÖ

Telephone No. +46 8 782 25 00

Form PCT/ISA/237 (cover sheet) (January 2004)

CORRECTED

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/NO2005/000012

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language, _____, which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/NO2005/000012

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	2-4,7-10	YES
	Claims	1,5-6	NO
Inventive step (IS)	Claims	3-4	YES
	Claims	1-2,5-10	NO
Industrial applicability (IA)	Claims	1-10	YES
	Claims		NO

2. Citations and explanations:

The subject-matter of the claims are directed to the second medical use of Agaricus blazei Murill to be used in the prevention and treatment of bacterial and non-bacterial infections as well as preventing allergy.

Reference is made to the following documents:

D1:Database WPI, AN 2003-785658 & KR 2003021096
D2: Osaki Yoshiko, Yakugaki Zasshi, jun 17, 1994, Vol.114, No.5, p342-350
D3: STN International, CAPLUS acc.no. 2001:461386.
D4 Sorimachi Kenji et al., Biosci. Biotechnol. Biochem., 2001, Vol.65, No 7, p. 1645-1647.
D5: EP 0413053
D6: WO01/85191

D5 discloses the use of a substance which is obtained from the mycelium of an edible mushroom, i.a. Agaricus blazei Murill, as a drug for preventing allergies(see page 5, lines

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

34-35, claims 1 and 9). D6 discloses a substance which is extracted from body tissues or mycelia tissues of Agaricus blazei which substance is an allergy cell activity inhibitor. It is thus known to use Agaricus blazei for preventing allergies. Claims 1, 5-6 are therefore not novel. Claims 7-10 are considered as being obvious modifications which are not considered to involve any inventive step.

It is not known from the prior art that Agaricus blazeii Murill is useful for treating bacterial infections caused by a parasite or a virus. As however there is no experimental data in the application showing that Agaricus blazei Murill is able to show an effect on non-bacterial infections such as infections caused by parasites or viruses, claim 2 is not sufficiently disclosed (see Box VIII). Claim 2 lacks an inventive step.

D1, D2, D3 and D4 disclose that the fungus Agaricus blazei has antibacterial effect but do not disclose that the fungus has activity against pneumococcus.

Claims 3-4 are novel and are considered to involve an inventive step.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/NO2005/000012

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawing or on the question whether the claim are fully supported by the description, are made:

Claims 1-2 and thereof dependent claims 6-10 are not supported by the description as required by Article 6 PCT, as their scope is broader than justified by the examples provided in the description. The reasons therefore are the following: in the application, technical data was provided showing effect against infections caused by pneumococci and against allergy. However no data was provided showing that Agaricus blazei Murill has effect against non-bacterial infections such as infections caused by parasites and virus.

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

REC'D 20 SEP 2005
WIPO PCT

To:
Oslo Patentkontor AS
Postboks 7007 M
N-0206 Oslo
Norge

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

	Date of mailing *(day/month/year)* 15 -09- 2005
Applicant's or agent's file reference 159885 TG-KR	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NO2005/000012	10.01.2005	12.01.2004

International Patent Classification (IPC) or both national classification and IPC
A61K 35/84 // A61P 31/00, A61P 37/08, A61P 33/00

Applicant
Geir Hetland

1. This opinion contains indications relating to the following items:
   - ☒ Box No. I Basis of the opinion
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☒ Box No. VIII Certain observations on the international application

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